Functional Gene Array-Based Analysis of Microbial Community Structure in a Gradient of Nitrate and Heavy Metal Contaminated Groundwaters

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ESPP2 Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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ABSTRACT

Six groundwater monitoring wells from the Field Research Center, site of the U.S. DOE Environmental Remediation Science Program (ERSP) at the Oak Ridge Reservation, Oak Ridge, TN, were selected to compose a gradient of pH (3.25 - 7.11), nitrate (1.2 - 41,790 mg/l) and heavy metal contamination (0 - 500 mg/l U; 0 - 39896 mg/l Tc). To determine the functional populations of bacteria present within the gradient, DNA was extracted from groundwater and analyzed with a functional gene array containing 2,006 gene probes for the detection of genes involved in metal-resistance, sulfate-reduction, contaminant degradation and carbon and nitrogen cycling. The signal intensities for each probe were used to measure community diversity and were correlated to the geochemical profile of each well. Diversity decreased in relation to the level of contamination within each well, and each community exhibited a different distribution of genes. Heatmaps of metal resistance genes and *nirK* and *nirS* genes indicate that highly contaminated wells had lower gene diversity, but greater signal intensity for detected genes. Wells with the highest sulfate concentrations had the greatest diversity and signal intensity for *dsrAB* genes. A greater number of carbon fixation genes (*cbbL*, *cbbM*) were detected than fermentation genes (FTHFS) in all wells. A variety of organic contaminant degradation genes were detected. Results of Mantel tests and canonical correspondence analysis indicate that nitrate, sulfate, pH, uranium and technetium have a significant ($p = 0.05$) effect on bacterial community structure. This study provides an overall picture of bacterial community structure in contaminated environments across many different functional genes and shows that diversity can vary widely in relation to the degree of contamination.

BACKGROUND

Figure 1. Photograph of the S-3 waste ponds before capping, at the Field Research Center (FRC) site of the U.S. Dept. Of Energy Environmental Remediation Science Program at Oak Ridge, TN. These ponds received a mixture of nitric acid, organic solvents, uranium, technetium and other heavy metals at rate of approximately 10^6 L of waste per year for 30 years before their closure in 1984.

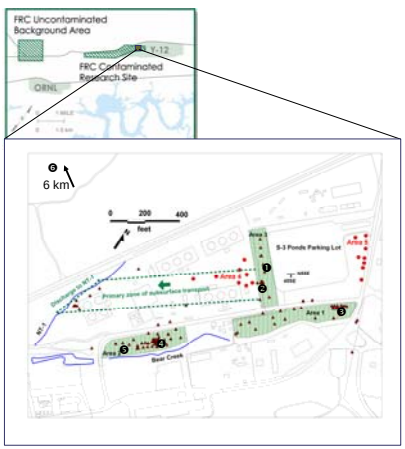

Figure 2. Map of the FRC background area and contaminated site. Inset area shows location of sampled wells. Numbers correspond to Table 1.

METHODS

- Six groundwater monitoring wells at the FRC were selected for sampling, to provide a gradient of contaminants. Groundwater was analyzed for metal and ion concentrations and pH.
- High molecular weight DNA was extracted from groundwater by freeze-thaw grinding and phenol/chloroform extraction.
- DNA was amplified by whole community rolling circle amplification (WCRCA) using a TempliPhi kit (GE Healthcare, Piscataway, NJ) and labeled with a Cy5 fluorescent dye.
- Labeled DNA was hybridized to the functional gene array in flow cells at 50ºC 10 h.
- Arrays were scanned and signal intensity for each probe was determined digitally by Imagene software (Biodiscovery Inc., Los Angeles, CA).
- Gene diversity analysis was performed using the hierarchical clustering algorithm in CLUSTER (Eisen *et al.*, 1998) and was visualized using TREEVIEW (http://rana.Stanford.edu/).
- Mantel tests (Mantel, 1967) for linking geochemistry and diversity were performed using PC-ORD v. 5.0 (McCune, B. and M. J. Mefford. 1999 MjM Software, Gleneden Beach, Oregon).
- Canonical correspondence analysis (CCA) (Hotelling, 1936) and partial CCA (pCCA) to partition variance in gene diversity to geochemical variables were performed using CANOCO (ter Braak, 1998).

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RESULTS

Table 1. Geochemical variables measured from each FRC monitoring well.

	Distance[a] (m)	Aluminum (mg/L)	Chloride (mg/L)	Lead (mg/L)	Nickel (mg/L)	Nitrate (mg/L)	pH	Selenium (mg/L)	Strontium (mg/L)	Sulfate (mg/L)	Technetium-99 (mg/L)	Uranium (mg/L)	Zinc (mg/L)
1. FW010	83	1120	875	25	18.9	41790	3.38	50	23.5	14	7190	500	12
2. FW024	95	3.84	298	0.2	0.28	8963	3.51	0.4	0.11	1116	39896.2	51.1	2.105
3. FW021	108	398	234.16	0.01	11.9	9451.52	3.25	0.03	1.83	2.5	30973.8	1.38	2.95
4. TPB16	312	0.62	21.97	0.1	0.05	67.35	5.86	0.2	0.46	123.35	13.2	0.79	0.04
5. FW003	356	0.4	183	0.2	0.1	1059	6.18	0.4	1.14	16	135	4	0.1
6. FW300	7019	0.2	1.12	0.1	0.05	1.2	7.11	0.2	0.1	6.21	0	0	0.05

[a]Distance measured from the center of the former S3 waste ponds to the monitoring well.

Figure 2. Relative abundance of functional genes detected by GeoChip in each well.

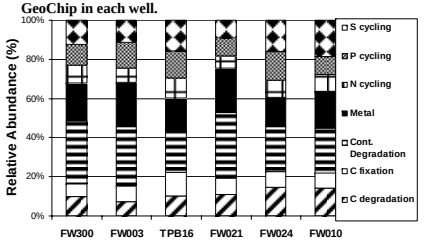

Figure 3. Functional gene heatmap analysis of gene diversity. Hierarchical cluster analysis of gene diversity based on hybridization signal intensities. Black indicates no hybridization above the background level, and red indicates signal intensity.

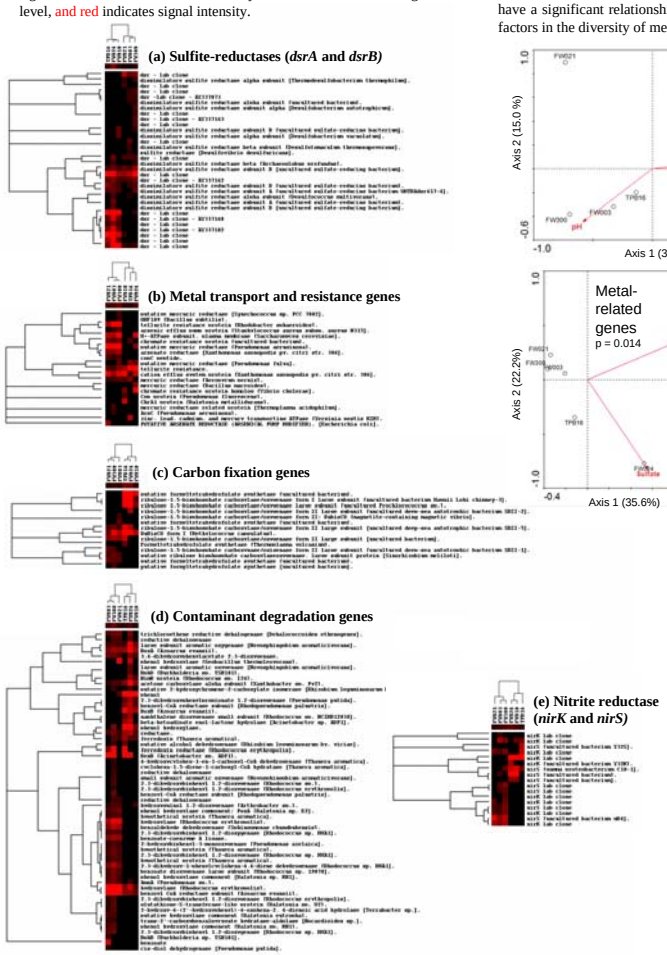

Figure 4. (a) Canonical Correspondence Analysis (CCA) and (b) Variance partitioning analysis of total genetic diversity and pH, uranium and sulfate levels in the wells. CCA of metal-related genes (c) and sulfite-reductase genes (*dsr*)(d) and uranium and sulfate levels. Sulfate concentrations have a significant relationship with total gene diversity. Sulfate and uranium appear to be important factors in the diversity of metal-related genes and sulfite-reductases.

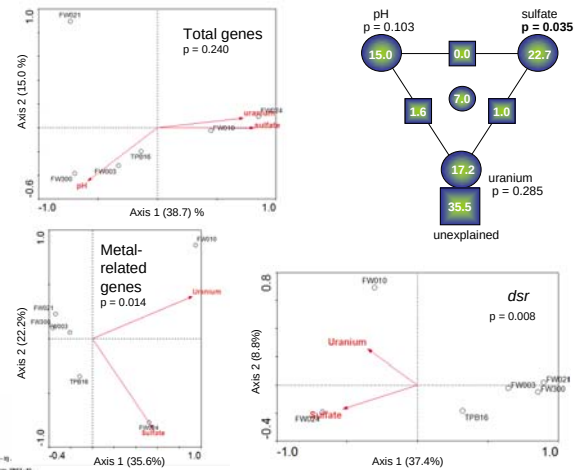

Table 2. Mantel Test results. Nitrate and Tc-99 concentrations, and the combination of nitrate, sulfate, U, Tc, and pH yield significant, negative correlations with the gene diversity observed.

Geochemical Variable	P-Value	R-value
Al	0.511	-0.171
Ba	0.487	-0.045
Ca	0.618	-0.028
Cd	0.434	-0.123
Cl	0.089	-0.197
K	0.467	-0.071
Mg	0.156	-0.204
Na	0.145	-0.167
Ni	0.325	-0.227
nitrate	**0.013**	**-0.253**
Pb	0.483	-0.013
pH	0.338	-0.123
Se	0.540	-0.002
Sr	0.484	-0.083
sulfate	0.101	0.288
Tc	**0.015**	**-0.289**
U	0.559	-0.009
Zn	0.341	-0.129
sulfate, nitrate, U, Tc, pH	**0.050**	**-0.220**
all geochemical variables	0.103	-0.217

SUMMARY

- Heatmap analysis show distinct communities in high and low level contaminant wells, with some common species. The majority of nitrite-reductase and sulfite-reductase genes are uncultured strains identified by molecular techniques.
- Mantel tests yield significant, negative correlations between the genetic diversity detected and concentrations of Tc-99 and nitrate, as well as the combination of sulfate, nitrate, U, Tc, and pH.
- pCCA analysis reveals that sulfate concentration can explain the greatest amount of variance in the gene diversity observed, followed by uranium and pH. Uranium and sulfate are also important factors in the diversity of metal-related genes and sulfite-reductases.
- Functional gene arrays such as the GeoChip can be powerful tools for correlating the geochemistry of an environment with the structure of the microbial community present in that environment.

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